Towards an integrated morphological and molecular WHO diagnosis of central nervous system tumors

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Abstract: Purpose of review: It is now fully clear that information on the molecular underpinnings of tumors of the central nervous system (CNS) can be used for a more robust characterization of at least selected neoplasms. During a meeting organized in Haarlem, The Netherlands, in May 2014, about 30 neuropathologists discussed how exactly molecular information could be incorporated in the routine classification of CNS tumors. Recent findings: This meeting laid the groundwork for an update of the WHO CNS tumor classification that integrates histopathological and molecular findings. Furthermore, a layered diagnostic approach was proposed that not only allows for integration of relevant molecular information in the pathological diagnosis, but also retains the option for rendering a diagnosis based on histopathological analysis alone. An integrated morphological and molecular definition of CNS tumors brings new challenges as well. For example, criteria for grading within molecularly defined categories of diffuse gliomas will require modification, and some tests used in clinical practice for the detection of molecular features, may provide false positive or false negative results. Summary: The evolving paradigm shift represents a major leap forward in the diagnosis of CNS tumors that will contribute substantially to optimizing interobserver reproducibility and clinico-pathological predictions.

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Towards an integrated morphological and molecular WHO diagnosis of central nervous system tumors: a paradigm shift

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Purpose of review
It is now fully clear that information on the molecular underpinnings of tumors of the central nervous system (CNS) can be used for a more robust characterization of at least selected neoplasms. During a meeting organized in Haarlem, The Netherlands, in May 2014, about 30 neuropathologists discussed how exactly molecular information could be incorporated in the routine classification of CNS tumors.

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Summary
The evolving paradigm shift represents a major leap forward in the diagnosis of CNS tumors that will contribute substantially to optimizing interobserver reproducibility and clinico-pathological predictions.

Keywords
brain tumor, grading, molecular

INTRODUCTION

The last two decades have witnessed seminal advances in our understanding of the molecular biology of tumors of the central nervous system (CNS). High throughput genetic profiling has facilitated the unbiased identification of genes differentially expressed in these tumors, which in turn has yielded new diagnostic biomarkers and potential therapeutic targets. Perhaps more surprising is the discovery that not only DNA-based alterations, but also epigenetic processes independent of the DNA sequence (histone modification, CpG island methylation, and dysregulation of DNA binding proteins) are co-conspirators in CNS tumor oncogenesis. The obvious question arises as to how these unprecedented advances should be incorporated in the routine classification of brain tumors. Although for many entities the traditional morphologic criteria of the WHO Classification of Tumors of the Central Nervous System [1] have stood the test of time, the genetic and epigenetic landscape of certain entities have shown a better correlation with prognosis. As a practical example, de-novo (primary) and secondary glioblastomas are histopathologically indistinguishable. However, at the genetic and epigenetic levels they show significant differences that potentially impact patient management [2,3]. At present, the classification of CNS tumors based on histopathological criteria alone

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KEY POINTS

- New classification schemes will be based on genetic and epigenetic signatures of brain tumors.
- A layered morphologic-molecular diagnostic approach will likely become the new diagnostic 'gold standard'.
- Pediatric brain tumors are biologically different from their adult counterparts and require different diagnostic criteria.

is undergoing a major revision in an attempt to integrate these rapidly emerging discoveries into daily practice. In May 2014, the International Society of Neuropathology (ISN)-Haarlem WHO's Next conference was convened to develop general practice guidelines for the neuropathology community [4] that would reflect the contribution of these advances and help lay the groundwork for the next WHO classification of CNS tumors, which is projected to be published in the first half of 2016. The aim of the present article is to draw attention to the ISN-Haarlem guidelines, to highlight several publications that illustrate key advances towards a more rational classification scheme for CNS tumors, and to indicate some aspects that need more attention, now that molecular characteristics are increasingly used for classification.

INTERNATIONAL SOCIETY OF NEUROPATHOLOGY-HAARLEM WHO'S NEXT GUIDELINES

In preparation for the Haarlem meeting, the participants, most of whom were neuropathologists with ample expertise in molecular diagnostics of tumors of the CNS, were asked to consider if and how molecular data should be used in the classification of brain tumors. Additional subquestions were posed that related to whether molecular analyses should be mandatory for entities with a defining molecular aberration, and if so, how the results should be reported. Various scenarios were discussed including how to report the histopathological findings when molecular analyses are ongoing and the preferred strategy for diagnostic sites where molecular diagnostic tools are unavailable. The consensus was that, when available, molecular genetic or epigenetic signatures should be routinely used to define previously ambiguous categories. Furthermore, it was concluded that CNS tumors in children often differ clinically and biologically from their histologically similar adult counterparts and therefore merit separate classification criteria. With respect to the reporting of imaging and clinical findings, the consensus was not to formerly integrate these aspects into the pathologic diagnosis. Another important conclusion from the Haarlem meeting was that improved classification of CNS tumors will depend on a successful partnership between biologists, clinicians, and neuropathologists.

Obviously, the application of characteristic molecular markers would prove especially useful to enhance diagnostic acumen when histopathological criteria are not sufficiently precise for recognition of clinically relevant subgroups and/or when tissue specimens are small or lack all the cardinal morphologic features of a specific entity. Atypical teratoid/rhabdoid tumor (AT/RT), a WHO grade IV embryonal tumor with rhabdoid features under the microscope, represents a test case. The consensus now is that 'canonical' AT/RTs carry a *SMARCB1* or (rarely) *SMARCA4* mutation, and that for the diagnosis of AT/RT one of these mutations should be demonstrated using molecular diagnostics. Alternatively, sound evidence for the presence of these mutations can be provided by demonstrating loss of, respectively, nuclear INI1 or BRG1 protein expression using immunohistochemistry. The final diagnosis would thus rest on the stepwise integration of morphologic with immunophenotypic and/or molecular data. In daily clinical practice an initial, descriptive histopathologic classification ('embryonal tumor with rhabdoid features') could be rendered, pending the integration of molecular analyses, which would be incorporated into a second report and lead to a final diagnosis of AT/RT (in case the molecular information supports this diagnosis) or of embryonal tumor with rhabdoid features (when no evidence of *SMARCB1* or *SMARCA4* mutation is found). Meanwhile, acknowledging that in molecularly 'bona fide' AT/RTs rhabdoid features may be lacking [5], using a combined morphological and molecular diagnostic approach, a high-grade malignant embryonal CNS tumor lacking rhabdoid features but without staining of tumor cell nuclei for INI1 or BRG1 as well, can still be diagnosed as AT/RT.

OLIGODENDROGLIOMA, ADVENT OF THE MOLECULAR AGE

For the group of diffuse gliomas (diffuse astrocytic tumors including glioblastomas, and oligodendroglial tumors), molecular diagnostics are very helpful for improved classification as well. Oligodendroglial tumors represent a forerunner for the role of molecular diagnosis in CNS tumor classification. The 2007 WHO classification recognizes two basic categories: pure oligodendroglioma and mixed oligoastrocytoma, which can be either 'low-grade' (grade II),

'anaplastic' (grade III) or, in the case of a mixed phenotype with necrosis, 'glioblastoma with oligodendroglial component' (grade IV) [1]. Although classic oligodendroglioma seldom poses a diagnostic dilemma, the lack of stringent criteria for diffuse gliomas with ambiguous features continues to erode the prognostic importance of assigning a WHO grade. Many neuropathologists have defaulted to the mixed oligoastrocytoma category when confronted with such cases. Not surprisingly, previous studies have shown poor interobserver reliability in capturing the morphologic distinction between these entities [6–8].

The recognition that diffuse gliomas with a classic oligodendroglial phenotype frequently show combined loss of the short arm of chromosome 1 and of the long arm of chromosome 19q (1p19q codeletion) represented the first milestone in identifying a potential biomarker [9]. Subsequently, the observation that the molecular signature was linked to a better prognosis [8,10] heralded a new era in brain tumor classification and fueled the debate as to whether oligodendroglioma should be defined by a molecular signature. A very recent, comprehensive review of the subject not only describes the differential diagnosis with occasional morphologic mimics such as pilocytic astrocytoma, glioneuronal tumors, and clear-cell ependymoma, but also emphasizes that with molecular diagnostics, the vast majority of diffuse gliomas in adults can be successfully separated into clinically relevant oligodendroglial or astrocytic tumors [11]. Consequently, the diagnosis of mixed oligoastrocytoma can be expected to largely disappear when adequate molecular analysis can be performed [12]. Standard markers that are recommended for use in a diagnostic algorithm include immunohistochemistry for the isocitrate dehydrogenase 1 (IDH1) R132H mutant protein and for ATRX (alpha thalassemia mental retardation syndrome X linked), a critical chromatin modifier, which often shows loss of nuclear expression in *IDH* mutant astrocytic tumors and but preservation in oligodendrogliomas. For tumors immunonegative for IDH1 R132H mutant protein, further molecular-based analyses are indicated to exclude less common other *IDH1* or *IDH2* mutations [13]. The consensus at the ISN-Haarlem meeting was that complete 1p19q codeletion indeed represents a valid surrogate marker for oligodendroglioma that substantially aids in establishment of prognosis and treatment selection. The term 'not otherwise specified' (NOS) designation could be added to the diagnosis in cases histologically diagnosed as oligodendroglioma without such molecular diagnostic support [4].

As detailed in the review article on oligodendroglioma [11], each method for assessment of the 1p19q codeletion status has its advantages and disadvantages. A pitfall for the frequently used fluorescent *in situ* hybridization (FISH) technique is that partial deletions of these chromosome arms are difficult to discriminate from complete 1p19q codeletion. Meanwhile, it is now clear that only the latter form of codeletion has a prognostically favorable impact. Another promising biomarker for improved classification of diffuse gliomas is the *TERT* promoter mutation. Interestingly, this mutation is mutually exclusive with ATRX mutations and is paradoxically seen in oligodendrogliomas and *IDH* wild-type high-grade gliomas [14].

GLIOBLASTOMA, A CHINK IN THE WALL

Until recently, the histopathological diagnosis provided the foundation for diagnosis, and importantly, a critical basis for therapy selection and outcome prediction for patients with a diffuse glioma. Current histopathological criteria for the diagnosis of glioblastoma, however, fail to adequately mirror the enormous biologic complexity of the entity. For the most part, morphologic subtypes such as giant cell glioblastoma, small cell glioblastoma, and gliosarcoma have not proven relevant for therapeutic or prognostic stratification. At the molecular level, with the exception of less frequent O6-methylguanine methyltransferase (*MGMT*) promoter methylation and epithelial growth factor receptor (*EGFR)* mutations, gliosarcoma is identical to glioblastoma without such sarcomatoid features. Similarly, almost all giant cell glioblastomas harbor *TP53* mutations, yet are clinically indistinguishable from conventional glioblastoma [15,16]. The concept of primary versus secondary glioblastoma was traditionally based on the recognition that glioblastoma can either arise *de novo* or through progression from a lower-grade diffuse astrocytoma. Indeed, such a dichotomy has now been validated by evidence of distinct molecular profiles, with secondary glioblastomas generally being *IDH* mutant and primary glioblastomas, *IDH* wild type. In addition, secondary glioblastomas often carry mutations in the *ATRX* and *TP53* genes, whereas primary glioblastomas typically show *EGFR* amplification, gain of chromosome 7, and loss of chromosome 10 [3,16]. Although the diagnosis of glioblastoma with an oligodendroglial component (GBM-O) was mentioned in the 2007 WHO classification, the preponderance of molecular evidence now dismisses GBM-O as a particular subtype of glioblastoma. After assessment of 1p19q status and of molecular makers such as EGFR amplification, gain of chromosome 7, and loss of chromosome 10, most cases can readily be assigned to either the anaplastic oligodendroglioma or glioblastoma category [11,17].

Aldape *et al.* [16] systematically reviewed the contribution of two decades of glioblastoma research, covering a range of topics that include genomic analysis, molecular pathogenesis, and transcriptional subtypes. Based on the observation that histologic grade III (anaplastic astrocytoma) and grade IV (glioblastoma) astrocytic tumors share key molecular chromosomal changes, the authors pose the provocative question of whether *IDH*-mutation status should replace traditional histopathological criteria [17]. Indeed, recent reports suggest that criteria for grading of diffuse gliomas need to be revised. For instance, the prognostic impact of assigning grade II versus grade III to an *IDH* mutant astrocytic tumor may not be that different any more [18,19]. Furthermore, most lower grade (i.e. WHO grade II or III) *IDH* wild-type diffuse astrocytomas appear to be biologically aggressive and behave as glioblastoma, even in the absence of microvascular proliferation and/or necrosis [16,20].

At the epigenetic level, promoter methylation of the *MGMT* gene, which can be assessed by several methodologies including methylation-specific PCR or pyrosequencing, is now part of the standard evaluation of high-grade astrocytomas. Methylation of the *MGMT* promoter is associated with a better response of glioblastoma to alkylating chemotherapy using temozolomide. Interestingly, most *IDH* mutated diffuse gliomas show *MGMT* promoter methylation in the context of a hypermethylated DNA status ('glioma CpG island methylated phenotype', G-CIMP). The G-CIMP status results from the 'oncometabolite' 2-hydroxyglutarate (2-HG) that is produced in excess because of the *IDH* mutation [15,21].

AGE MATTERS

Comprehensive genetic and epigenetic studies have yielded compelling evidence that pediatric brain tumors are fundamentally different from morphologically similar tumors in adults. At the top of the list of clinically actionable discoveries in pediatric CNS tumors is the molecular subclassification of medulloblastoma, which include the SHH and WNT pathways [22,23]. Pilocytic astrocytoma, a far more common childhood brain tumor, is now considered a one-pathway disease involving the MAPK pathway. Frequent underlying molecular aberrations include a tandem duplication on chromosome 7 resulting in the *KIAA1549–BRAF* fusion protein and *BRAF* V600E mutation [24]. Other notable discoveries in pediatric gliomas include the lysine to methionine (K27M) substitution in histones H3.1 and H3.3 in over 80% of diffuse midline gliomas including diffuse intrinsic pontine gliomas. In contrast to adults, *IDH1* or *IDH2* mutations and/or 1p19q codeletion are virtually absent in pediatric diffuse gliomas [25–27]. The rare tumors previously designated embryonal tumor with abundant neuropil and true rosettes, ependymoblastoma, or medulloepithelioma were recently shown to share molecular similarity and to in fact comprise a single clinicopathological entity. This entity is now tentatively grouped under the term 'embryonal tumor with multilayered rosettes', which can now be reliably diagnosed by combined LIN28A immunohistochemistry and FISH analysis of the 19q13.42 locus [28,29]. Advances have even been made in the molecular classification and prognostic stratification of ependymomas, which are long overdue, given the inconsistent results that have plagued histological grading [30].

CONCLUSION

The ISN-Haarlem WHO's Next meeting has laid the groundwork for an evolving paradigm of CNS tumor classification that integrates histopathological and molecular results in a layered fashion. This layered diagnostic approach not only allows for the integration of relevant molecular information in the pathological diagnosis but also retains the option of a histopathological diagnosis for centers/countries where molecular diagnostics is not available. Meanwhile, it is fully clear that the expanding catalogue of molecular markers has at the same time contributed to the elucidation of the pathogenesis of CNS neoplasms and to improved diagnostic stratification. It can be expected that the rapid pace of discoveries in (CNS) tumor biology and genetics that have marked the last decade will continue to refine diagnostic strategies. Hopefully, translation of these discoveries will facilitate the development and selection of more effective therapies for the patients suffering from CNS tumors as well.

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Conflicts of interest

There are no conflicts of interest.

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